追加資料1

血液事業部会運営委員会委員　岡田　義昭

XMRVに関する文献報告（続報）（平成２３年９月２８日）

文献番号	文献名	報告国	要約
1	Simmons G,Glynn S A,Komaroff A L, et al.,Science 22 Sep. 2011 Failure to Confirm XMRV/MLVs in the Blood of Patients with Chronic Fatigue Syndrome: A Multi-Laboratory Study	米国	慢性疲労症候群（CFS）とマウスレトロウイルスとの関係が論争されている。そこで、これまでXMRV陽性とされた15検体（内CFS患者由来14検体）と15の陰性検体を集め、9施設でブラインド形式でNAT、ウイルス培養、抗体の3つの方法でXMRVを検出した。NATは7つの施設で血漿、PBMC、全血を用いて実施され、6施設では全て陰性であったが、1施設のみが過去に陽性と判断された1検体が2回の試験で1回陽性となった。しかし、その施設では陰性コントロールが2検体陽性となっていた。また、抗体検出では、組み換え蛋白、又はウイルスから精製したタンパクを抗原に用いた2施設では全ての検体から抗体は検出されなかった。一方、SFFVウイルスの抗原を発現している細胞を用いてフリーサイトメーターでXMRVの抗体を検出した2施設では陰性コントロールで15検体中8例及び6例が抗体陽性、過去に陽性とされた15検体では5例と10例が抗体陽性と判断された。この2施設では、再現性に一致が見られなく、陰性コントロールと陽性検体とに有意の差が認められなかった。以上の結果は、現在の試験法では血液からXMRV/MLVは再現的に検出されないこと、及び、血液のスクリーニングを実施する正当性がないことを示している。
2	Silverman R H,Das Gupta J ,Lombardi V C, et al., Science 2011, Sep. 22 Partial Retraction to "Detection of an Infectious Retrovirus,XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome"	米国	Science 326,585(2009)に報告した慢性疲労症候群患者（CFS）の末梢血単核球 DNAからのXMRV遺伝子検出に関し、XMRVのエンベロープ遺伝子、XMRVが挿入されていたプラスミドのネオマイシン遺伝子、同プラスミドにあるCMVのプロモーターと挿入されているXMRV遺伝子との結合部分、計3箇所をそれぞれ増幅するプライマーを用いてPCRを再度実施した。15例のCFS患者のうち6例から envが検出された。しかし、陽性となった検体は同時にプラスミド由来の2つの遺伝子も 陽性であった。塩基配列の解析からXMRVを組み込まれたVP62（プラスミドの名前）由来と考えられた。以上から、以前報告した論文のデーターから該当する部分を撤回する。

Sciencexpress Report

Failure to Confirm XMRV/MLVs in the Blood of Patients with Chronic Fatigue Syndrome: A Multi-Laboratory Study

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Murine leukemia viruses (MLV), including xenotropic-MLV-related virus (XMRV), have been controversially linked to chronic fatigue syndrome (CFS). To explore this issue in greater depth, we compiled coded replicate samples of blood from 15 subjects previously reported to be XMRV/MLV-positive (14 with CFS) and from 15 healthy donors previously determined to be negative for the viruses. These samples were distributed in a blinded fashion to nine laboratories which performed assays designed to detect XMRV/MLV nucleic acid, virus replication, and antibody. Only two laboratories reported evidence of XMRV/MLVs; however, replicate sample results showed disagreement and reactivity was similar among CFS subjects and negative controls. These results indicate that current assays do not reproducibly detect XMRV/MLV in blood samples and that blood donor screening is not warranted.

Novel murine leukemia virus (MLV)-like sequences were identified in, and implicated as a potential infectious cause of, human prostate cancer in 2006 (*1*). These sequences appeared to be closely related to xenotropic MLV (X-MLV) and were termed X-MLV-related virus or XMRV. In 2009, similar viral sequences were identified in a cohort of patients with chronic fatigue syndrome (CFS) (*2*). In that study XMRV could be directly cultured from both peripheral blood mononuclear cells (PBMC) and plasma from the majority of patients with CFS, and XMRV sequences were detected by PCR and RT-PCR (*2*, *3*). Furthermore, evidence of an immune response to MLVs was observed in patient plasma (*2*, *3*). In an independent study, other patients with CFS were reported to harbor MLV-related virus sequences, but not XMRV, in PBMC and plasma (*4*). These sequences were derived from viruses resembling polytropic MLVs (P-MLV), rather than X-MLV. Importantly, both studies identified XMRV/P-MLV in the majority (67 to 86%) of patients with CFS but also in substantial numbers of healthy controls including blood donors (4-7%) (*2*, *4*).

Subsequent studies cast doubt on the association between XMRV/P-MLVs and CFS, and indeed on the detection of XMRV/P-MLVs in human populations (reviewed in (*5*)). Many, although not all (*6*, *7*), of these negative studies focused on nucleic acid detection and/or serology and did not include cell culture assays for virus (*8*–*11*). Several additional findings raised uncertainty about the high rates of XMRV/P-MLV in patients with CFS that had been described in the two seminal papers: (i) clinical samples and PCR reagents were found to be contaminated by XMRV and mouse DNA containing endogenous MLVs (*12*); (ii) XMRV and P-MLV lack the sequence diversity that would be expected to arise following transmission, infection, and repeated cycles of replication of a retrovirus in humans (*13*, *14*), and (iii) evidence was presented which strongly suggested that XMRV originated in the early 1990s by recombination of endogenous

MLVs following serial passage of a human prostate xenograft in laboratory mice (*15*). It was postulated that this laboratory passage resulted in the generation of several prostate cancer cell lines harboring integrated XMRV sequences that produced high levels of infectious virions. These XMRV-infected cell lines were subsequently widely disseminated and likely produced inadvertent XMRV contamination of laboratories and reagents (*15*).

We report here the results of a comprehensive study where multiple laboratories analyzed the same blood samples for XMRV/P-MLV. These blood samples, which were drawn from persons who were previously reported to be XMRV- (*2*) or P-MLV-positive (*4*) and from blood donors who previously tested negative for XMRV, were aliquoted into replicate tubes and assembled into coded panels together with replicates of experimentally prepared positive control samples. The testing was performed fully blinded to remove bias. These samples were tested by nine laboratories using highly sensitive and previously validated nucleic acid, serological and culture assays (tables S1 to S5) for XMRV and other MLVs (*16*). The two laboratories that had previously found an association for the MLVs with CFS participated in this study (*2*, *4*). All nine laboratories used XMRV/P-MLV nucleic acid amplification testing (NAT), serological and/or culture assays of their own choosing which were incorporated into parallel or serial testing algorithms to generate final results. The majority of laboratories included assays to detect murine DNA contamination either on all samples or on all NAT positive samples.

Fourteen patients with CFS, together with one person reporting contact with a CFS patient [described in supporting online material (*17*)], all of whom were previously reported to be XMRV/P-MLV-positive by at least one method (table S6) were enrolled into the study at two clinical sites using IRB-approved protocols and consents (referred to as the XMRV/P-MLV cohorts henceforth). Per study protocol, none of the 15 subjects were on antiretrovirals, but several later disclosed that they were taking other antivirals (e.g., valacyclovir) and two were on immunosuppressive medications (the latter are indicated in table S6). In the case of the P-MLV-like viruses described by Lo and colleagues (*4*), only PCR detection had been performed in the original study; four of five patients enrolled into the current study were reported to be P-MLV reactive on the archived samples from the original cohort study and on a second sample collected 15 years later (2010) whereas one patient was PCR-positive only on the original archived sample (*4*). The Whittemore Peterson Institute (WPI) patient cohort was more intensively characterized as positive by PCR, serology and/or culture, although none of the study subjects tested positive in all assays at all time points (table S6).

To minimize introduction of potential contaminants, we took extensive precautionary measures during the collection of specimens and the laboratory processing of blood and preparation of sample aliquots (*17*). Blood specimens were collected by independent phlebotomists, shipped to the central laboratory (*17*), and processed into coded PBMC, plasma and whole blood (WB) aliquots. Similarly, fifteen control specimens from blood donors (n=12) or laboratory controls (n=3) that had been established as negative for XMRV and MLVs by PCR, serology and culture by multiple laboratories, were collected, processed and aliquoted in parallel (*17*). Finally, a separate facility in the central laboratory prepared and characterized stocks of the XMRV-infected human cell line 22Rv1 (*15*, *18*) and supernatant, which were used to spike samples to create a set of low-level positive controls (*17*).

A total of eleven NAT, five serology and three culture assays were performed on the samples (*17*). The WPI laboratory did not report culture assay results because their target cells had become contaminated with mycoplasma. Other than this, all sites reported results on all distributed and coded sample aliquots to the central laboratory. The results were then decoded and compiled into analysis datasets specific to the panels.

Few positive NAT results were reported, other than on the coded spiked positive control replicate aliquots (table 1) (table S7). Six of seven laboratories that performed NAT on three sample types (plasma, PBMC and WB) reported no positive result for coded clinical samples (XMRV/P-MLV cohorts or negative controls), whereas these laboratories detected XMRV in 100% of the spiked controls (table 1). These laboratories included those that employed the most sensitive XMRV/P-MLV assays available, based on our previous blinded analytical sensitivity performance study (*16*). Of particular note, the FDA/Lo laboratory failed to detect MLV-like sequences using the same nested PCR assay as previously published, in either the known negative controls or in the XMRV/P-MLV cohort samples. The samples scored as negative by this laboratory included the replicate samples from five patients with CFS reported as P-MLV positive in their previous study, four of whom had also tested positive on a second specimen collected over a decade after the archived CFS cohort panel (*4*).

The only positive NAT results on some of the replicates from clinical samples were reported by WPI. The WPI assays appeared less sensitive than those used by the other laboratories, based on the fact that only 3 of 5 plasma and 4 of 5 PBMC-spiked positive control replicates were scored as positive by WPI (table 1) (table S7). However, two plasma clinical aliquots were reported as positive in the WPI nested RT-PCR *gag* assay. These samples were from two different negative controls, and only one out of the three replicates was positive in each case. Sequencing of the excised bands revealed 1-3 base changes compared to XMRV derived from 22Rv1 (supporting online text). A clinical PBMC sample, derived from one of the nine WPI CFS patients, was also positive in WPI's nested *gag* PCR assay. However, only one of two PBMC replicates for this individual was positive, and all replicates of plasma and WB from this patient were reported as negative by WPI. All positive samples tested negative for mouse DNA contamination as assessed by mouse mitochondrial DNA PCR (*4*). Reactivity rates did not significantly differ between samples from negative controls and the XMRV/P-MLV cohorts (p >0.05) (supporting online text, table S10).

In the initial study, Lombardi et al. reported that the most effective and consistent method of determining whether an individual was XMRV-positive was by isolation of replication-competent virus through co-culture of target prostate cell lines with either patient PBMCs or plasma (*2*, *3*). Although culture results were not reported by WPI in the present study, the NCI/Ruscetti laboratory also successfully performed virus culture using both plasma and PBMC in the Lombardi et al. study (*2*, *3*). Additionally, virus culture was performed by the FDA/Hewlett laboratory, which used two methods, one of which (LNCaP cell culture) was established in their laboratory for this study based on WPI procedures and on-site training by the lead investigators from the WPI and NCI/Ruscetti laboratories, and hence viral culture in this laboratory would be expected to have equivalent sensitivity to the culture method used by Lombardi et al. (*17*). Both laboratories successfully detected all five replicates of the spiked positive controls (~10^6 RNA copies/ml). However, while neither of the FDA/Hewlett assays detected confirmed positive cultures in the 30 coded clinical aliquots, the NCI/Ruscetti laboratory reported nine aliquots as positive (table 1, 2). Six of the positive results were from negative control samples (40% positive rate); these six subjects/samples had previously been pedigreed by the same laboratory as culture-negative (*17*). In contrast, only three (20%) of the 15 XMRV/P-MLV-cohort subjects (including ten subjects who had previously been found to be culture-positive by the WPI and NCI/Ruscetti laboratories) tested positive in the coded panel (table S1). There was no significant difference between the rate of reported positive culture results among negative controls and the XMRV/P-MLV cohort subjects (p-value = 0.43, table S8).

Finally, serology was performed by four laboratories (*17*). Although plasma with human antibodies to XMRV/P-MLVs was not available to produce spiked controls for serology, all four laboratories performed their own internal controls (*17*). Three assays --a Western blot test using purified XMRV (CDC) (*19*) and two chemiluminescent immunoassays using recombinant XMRV gp70 and p15E (Abbott Diagnostics) (*20*)-- failed to detect positive results for any of the coded replicates prepared from the 30 clinical samples. A flow cytometry-based serologic assay run by two laboratories (NCI/Ruscetti and WPI), utilizing mouse cells expressing the spleen focus-forming virus (SFFV) envelope as employed in the original Lombardi et al. study, reported a number of positive results on samples from both the XMRV/P-MLV cohorts and the negative-plasma controls. The NCI/Ruscetti laboratory reported 13 positive samples, including eight (53%) from 15 known negatives and five (33%) from 15 XMRV/P-MLV cohort subjects (table 1) (table 2). None of the positive results from the XMRV/P-MLV cohorts or controls were reported for more than one of the uniquely coded replicates, despite the fact that every sample was represented in the panel in duplicate or triplicate (table 2). There was no significant difference between the proportions of negative controls and XMRV/P-MLV cohort subjects identified as serology-positive (p-values >0.20 regardless of how positivity was defined [supporting online text, table S9]).

Among all serologic replicates tested, the WPI detected 22 positives, including 10 reactive results among the negative controls, and six each in the subjects previously reported as positive by WPI and by FDA/Lo (table 1) (table S7). Three of the six known negative controls with a positive serology result had at least two of three replicates positive (table 2). All five patients previously identified as P-MLV positive by FDA/Lo had a replicate called serology positive, but only one had both replicates reported as positive. Similarly for the 10 subjects previously identified as XMRV positive by WPI, four subjects had one of two replicates reported as serology positive, while both replicates from one patient were reported positive (table 2). There was no significant difference in the rates of positive WPI serology results between negative controls and XMRV/P-MLV cohort subjects (p-value = 0.27). There was no statistical agreement between the samples reported as serology positive by the NCI/Ruscetti and WPI laboratories, despite the fact that they used similar assays (supporting online text, tables S9, S10). Kappa values were calculated for each criterion and for all subjects combined using standard procedures (*17*, *21*). The Kappa values for level of agreement of results between these two laboratories ranged from -0.20 for WPI XMRV/P-MLV-positive subjects (no agreement) to 0.21 for all negative controls combined (fair agreement). However, the most telling Kappa value between the WPI and NCI/Ruscetti serology results is the one computed for all subjects combined, which is 0.01 indicating no agreement.

In summary, our study demonstrates that no XMRV/P-MLV assay in any of the nine participating laboratories could reproducibly detect XMRV/P-MLV in fifteen subjects (fourteen with CFS) who had previously been reported as XMRV/P-MLV-infected usually at multiple time points and often by multiple assays (*2*, *4*). The two laboratories (WPI and NCI/Ruscetti labs) that reported positive results in this study reported similar rates of reactivity among XMRV/P-MLV subjects and known negative control donor samples. The results from both laboratories were inconsistent when their assays were performed in parallel on replicate sample aliquots derived from individual subject specimens. There was also no agreement of reactivity when comparing results between these two laboratories for the 30 blinded XMRV/P-MLV cohorts and control samples. In contrast, assays developed by FDA (Lo and Hewlett), CDC, NCI/DRP, Abbott Diagnostics, Abbott Molecular and Gen-Probe, all of which have been designed to detect XMRV and relevant MLVs with high sensitivity and specificity, failed to detect evidence of viral infection in any of the previously positive subjects, including CFS patients, or negative control specimens represented in the study.

Altogether, 15 XMRV/P-MLV cohort subjects were represented in this study, the maximum number of subjects who could be recruited by the cohort investigators (*2*, *4*). Since most patients were selected based on having previously tested positive for XMRV/P-MLV 1-3 years ago, it is possible that levels of viremia and/or antibody could have waned by the time samples were drawn in our study; however, this is contradictory to Lo et al.'s finding that 4 of 5 patients retested positive 15 years later (*4*). The inconsistent reactive results from the two laboratories that previously reported detection of XMRV (NCI/Ruscetti and WPI) and the negative results from all other laboratories, including the laboratory that previously reported detection of P-MLV (FDA/Lo), strongly suggest that the positive reactivity in this study represents false positive results due to assay non-specificity or cross-reactivity (e.g. to other endogenous or exogenous retroviruses). However, we cannot definitively exclude the possibility that the levels of XMRV/P-MLV markers in blood may be at or below the limit of detection of all assays and/or fluctuate over time as recently described in experimentally infected macaque studies (*22*).

Based on these findings, we conclude that currently available XMRV/P-MLV assays, including the assays employed by the three participating laboratories that previously reported positive results on samples from CFS patients and controls (*2*, *4*), cannot reproducibly detect direct virus markers (RNA, DNA, or culture) or specific antibodies in blood samples from subjects previously characterized as XMRV/P-MLV positive (all but one with a diagnosis of CFS) or healthy blood donors. Finally, our findings are reassuring with respect to blood safety and indicate that routine blood donor screening for XMRV/P-MLV is not warranted at this time.

References and Notes

1. A. Urisman *et al.*, Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* **2**, e25 (2006).
2. V. C. Lombardi *et al.*, Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* **326**, 585 (2009).
3. J. A. Mikovits, V. C. Lombardi, M. A. Pfost, K. S. Hagen, F. W. Ruscetti, Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Virulence* **1**, 386 (2010).
4. S. C. Lo *et al.*, Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. *Proc Natl Acad Sci U S A* **107**, 15874 (2010).
5. F. J. van Kuppeveld, J. W. van der Meer, XMRV and CFS-the sad end of a story. *Lancet*, (2011), doi:10.1016/S0140-6736(11)60899-4.
6. K. Knox *et al.*, No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. *Science* **333**, 94 (2011).
7. C. H. Shin *et al.*, Absence of XMRV retrovirus and other murine leukemia virus-related viruses in patients with chronic fatigue syndrome. *J Virol* **85**, 7195 (2011).
8. O. Erlwein *et al.*, Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. *PLoS One* **5**, e8519 (2010).
9. P. Hong, J. Li, Y. Li, Failure to detect Xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome. *Virol J* **7**, 224 (2010).
10. E. R. Gray *et al.*, No evidence of XMRV or related retroviruses in a London HIV-1-positive patient cohort. *PLoS One* **6**, e18096 (2011).
11. S. Tang *et al.*, Absence of detectable xenotropic murine leukemia virus-related virus in plasma or peripheral blood mononuclear cells of human immunodeficiency virus Type 1-infected blood donors or individuals in Africa. *Transfusion* **51**, 463 (2011).
12. R. A. Smith, Contamination of clinical specimens with MLV-encoding nucleic acids: implications for XMRV and other candidate human retroviruses. *Retrovirology* **7**, 112 (2010).
13. S. Hue *et al.*, Disease-associated XMRV sequences are consistent with laboratory contamination. *Retrovirology* **7**, 111 (2010).
14. A. Katzourakis, S. Hue, P. Kellam, G. J. Towers, Phylogenetic analysis of MLV sequences from longitudinally sampled Chronic Fatigue Syndrome patients suggests PCR contamination rather than viral evolution. *J Virol.* (2011). [Epub ahead of print] doi:10.1128/JVI.00827-11.

15. T. Paprotka *et al.*, Recombinant origin of the retrovirus XMRV. *Science* **333**, 97 (2011).
16. G. Simmons *et al.*, The Blood Xenotropic Murine Leukemia Virus-Related Virus Scientific Research Working Group: mission, progress, and plans. *Transfusion* **51**, 643 (2011).
17. Materials and methods are available as supporting material on Science Online.
18. E. C. Knouf *et al.*, Multiple integrated copies and high-level production of the human retrovirus XMRV (xenotropic murine leukemia virus-related virus) from 22Rv1 prostate carcinoma cells. *J Virol* **83**, 7353 (2009).
19. B. C. Satterfield *et al.*, Serologic and PCR testing of persons with chronic fatigue syndrome in the United States shows no association with xenotropic or polytropic murine leukemia virus-related viruses. *Retrovirology* **8**, 12 (2011).
20. X. Qiu *et al.*, Characterization of antibodies elicited by XMRV infection and development of immunoassays useful for epidemiologic studies. *Retrovirology* **7**, 68 (2010).
21. J. Cohen, A coefficient of agreement for nominal scales. *Educ Psychol Meas* **20**, 37 (1960).
22. N. Onlamoon *et al.*, Infection, viral dissemination, and antibody responses of rhesus macaques exposed to the human gammaretrovirus XMRV. *J Virol* **85**, 4547 (2011).
23. K. Fukuda *et al.*, The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* **121**, 953 (1994).
24. B. Dong *et al.*, An infectious retrovirus susceptible to an IFN antiviral pathway from human prostate tumors. *Proc Natl Acad Sci U S A* **104**, 1655 (2007).
25. R. Schlaberg, D. J. Choe, K. R. Brown, H. M. Thaker, I. R. Singh, XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. *Proc Natl Acad Sci U S A* **106**, 16351 (2009).
26. W. M. Switzer, H. Jia, H. Zheng, S. Tang, W. Heneine, No association of xenotropic murine leukemia virus-related viruses with prostate cancer. *PLoS One* **6**, e19065 (2011).
27. O. Erlwein *et al.*, DNA extraction columns contaminated with murine sequences. *PLoS One* **6**, e23484 (2011).
28. P. W. Tuke, K. I. Tettmar, A. Tamuri, J. P. Stoye, R. S. Tedder, PCR master mixes harbour murine DNA sequences. Caveat emptor! *PLoS One* **6**, e19953 (2011).
29. W. M. Switzer *et al.*, Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. *Retrovirology* **7**, 57 (2010).
30. B. Oakes *et al.*, Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. *Retrovirology* **7**, 109 (2010).
31. S. Palmer *et al.*, New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* **41**, 4531 (2003).
32. K. S. Sfanos *et al.*, Identification of replication competent murine gammaretroviruses in commonly used prostate cancer cell lines. *PLoS One* **6**, e20874 (2011).
33. Y. A. Zhang *et al.*, Frequent detection of infectious xenotropic murine leukemia virus (XMLV) in human cultures established from mouse xenografts. *Cancer Biol Ther* **12**, (2011).
34. C. Chaipan *et al.*, Severe restriction of xenotropic murine leukemia virus-related virus replication and spread in cultured human peripheral blood mononuclear cells. *J Virol* **85**, 4888 (May, 2011).
35. A. Agresti, *Categorical Data Analysis*. (Wiley, ed. 2nd, 2002).

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screening assays for pathogen nucleic acids. S. K. is a paid consultant to Novartis Diagnostics, a distributor of blood donor screening assays, and to Cerus Corporation, a manufacturer of pathogen inactivation systems for blood components. The Whittemore Peterson Institute has filed patent applications related to methods of testing XMRVs and variants in blood. Abbott Laboratories has filed patent applications relating to detection of XMRV using immunoassays and molecular-based assays. Gen-Probe has filed patent applications relating to the assays they performed in this paper.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1213841/DC1
Materials and Methods
SOM Text
Tables S1 to S10
References (*23*–*35*)
Appendix S1

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Table 1. All XMRV/P-MLV assay results from all laboratories.

Test	**Laboratory**	**Sample type**			
		Negative Controls*	WPI XMRV/P-MLV Subjects*	Lo et al. XMRV/P-MLV Subjects*	Spiked Controls*
NAT/Plasma	Abbott-M†	0/15	0/10	0/5	**5/5**
	CDC	0/15	0/10	0/5	**5/5**
	FDA/Lo	0/15	0/10	0/5	**5/5**
	FDA/Hewlett	0/15	0/10	0/5	**5/5**
	Gen-Probe	0/15	0/10	0/5	**5/5**
	NCI/DRP	0/15	0/10	0/5	**5/5**
	WPI	**2/15‡**	0/10	0/5	**3/5**
NAT/PBMC	Abbott-M	0/3	0/10	0/5	**5/5**
	CDC	0/3	0/10	0/5	**5/5**
	FDA/Lo	0/3	0/10	0/5	**5/5**
	FDA/Hewlett	0/3	0/10	0/5	**5/5**
	Gen-Probe	0/3	0/10	0/5	**5/5**
	NCI/DRP	0/3	0/10	0/5	**5/5**
	WPI	0/3	**1/10‡**	0/5	**4/5**
NAT/WB	Abbott-M	0/15	0/10	0/5	**5/5**
	CDC	0/15	0/10	0/5	**5/5**
	FDA/Lo	0/15	0/10	0/5	**5/5**
	FDA/Hewlett	0/15	0/10	0/5	**5/5**
	Gen-Probe	0/15	0/10	0/5	**5/5**
	NCI/DRP	0/15	0/10	0/5	**5/5**
	WPI	0/15	0/10	0/5	**5/5**
Culture	FDA/Hewlett	0/15	0/10	0/5	**5/5**
	NCI/Ruscetti	**6/15**	**3/10**‡	0/5	**5/5**
Serology	Abbott-D	0/15	0/10	0/5	N/A
	CDC	0/15	0/10	0/5	N/A
	NCI/Ruscetti	**8/15**	**3/10**	**2/5‡**	N/A
	WPI	**6/15**	**5/10**	**5/5‡**	N/A

*Number positive/number tested. A single reactive replicate out of 1, 2, or 3 tested for a given individual was considered positive

†Abbott-M, Abbott Molecular; Abbott-D, Abbott Diagnostics; WB, whole blood; N/A, not applicable

‡No significant association was seen when the reactivity rates of control negatives and XMRV/P-MLV cohort subjects were compared [*P* values are discussed (supporting online material text)]

Table 2. Results of replicates for assays with positive results (number reactive/number replicates tested).

Sample Type	**Subject**	**Assay** **WPI NAT/Plasma**	**WPI NAT/PBMC**	**WPI Serology**	**NCI/Ruscetti Serology**	**NCI/Ruscetti Culture**
Negative Controls						
	1	0/1	0/2	0/2	0/2	0/1
	2	0/1	0/1	0/2	0/2	**1/1**
	3	0/1	0/1	**2/2**	**1/2**	0/1
	4	0/3	NT*	**1/3**	**1/3**	0/1
	5	0/3	NT	0/3	0/3	0/1
	6	**1/3**	NT	0/3	**1/3**	0/1
	7	0/3	NT	0/3	0/3	0/1
	8	0/3	NT	**2/3**	0/3	0/1
	9	0/3	NT	**3/3**	**1/3**	**1/1**
	10	0/3	NT	0/3	**1/3**	**1/1**
	11	0/3	NT	**1/3**	**1/3**	0/1
	12	0/3	NT	**1/3**	0/3	**1/1**
	13	0/3	NT	0/3	**1/3**	**1/1**
	14	**1/3**	NT	0/3	**1/3**	**1/1**
	15	0/3	NT	0/3	0/3	0/1
				†		
WPI XMRV/P-MLV Subjects						
	1	0/1	0/2	0/2	**1/2**	0/1
	2	0/1	0/1	**1/2**	0/2	0/1
	3	0/1	0/1	**1/2**	0/2	0/1
	4	0/2	0/2	**2/2**	0/2	**1/1**
	5	0/1	**1/2**	0/2	0/2	**1/1**
	6	0/1	0/2	0/2	0/2	0/1
	7	0/2	0/2	0/2	0/2	0/1
	8	0/1	0/2	**1/2**	**1/2**	0/1
	9	0/1	0/3	**1/2**	0/2	0/1
	10	0/1	0/2	0/2	**1/2**	**1/1**
				‡		
Lo et al. XMRV/P-MLV Subjects						
	1	0/3	0/2	**1/2**	0/2	0/1
	2	0/3	0/2	**1/2**	0/2	0/1
	3	0/3	0/2	**1/2**	**1/2**	0/1
	4	0/3	0/2	**1/2**	**1/2**	0/1
	5	0/3	0/1	**2/2**	0/2	0/1
				§, □		
Spiked Controls						
	1	**3/5**	**4/5**	NT	NT	**5/5**

*NT, not tested.

†The kappa for the serology for the negative controls between NCI/Ruscetti and WPI is 0.21.

‡The kappa for the serology for the WPI XMRV/P-MLV subjects between NCI/Ruscetti and WPI is –0.20.

§The kappa for the serology for the Lo et al. XMRV/P-MLV subjects between NCI/Ruscetti and WPI is 0.00.

□The kappa for the serology for all cohort subjects between NCI/Ruscetti and WPI is –0.08.

Sciencexpress Letter

Partial Retraction

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In our 23 October 2009 Report, "Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome" (*1*), two of the coauthors, Silverman and Das Gupta, analyzed DNA samples from chronic fatigue syndrome (CFS) patients and healthy controls. A reexamination by Silverman and Das Gupta of the samples they used shows that some of the CFS peripheral blood mononuclear cell (PBMC) DNA preparations are contaminated with XMRV plasmid DNA (*2*). The following figures and table were based on the contaminated data: Figure 1, single-round PCR detection of XMRV sequences in CFS PBMC DNA samples; table S1, XMRV sequences previously attributed to CFS patients; and figure S2, the phylogenetic analysis of those sequences. Therefore, we are retracting those figures and table.

References

1. V. C. Lombardi , F. W. Ruscetti, J. Das Gupta, M. A. Pfost, K. S. Hagen, D. L. Peterson, S. K. Ruscetti, R. K. Bagni, C. Petrow-Sadowski, B. Gold, M. Dean, R. H. Silverman, J. A. Mikovits, *Science* **326**, 585 (2009).
2. Supporting online material showing the reanalysis is available at www.sciencemag.org/cgi/content/full/science.1212182/DC1

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1212182/DC1

Materials and Methods

SOM Text

Figs. S1 to S4

Tables S1 and S2

References

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